NMR Spectroscopy

DOI: 10.1002/anie.200902398

On the Treatment of Conformational Flexibility when Using Residual Dipolar Couplings for Structure Determination**

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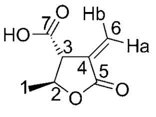
The determination of the three-dimensional structure of organic and biomolecular compounds by NMR spectroscopy usually involves the measurement of ^3J coupling constants,[1] NOEs,[2] and cross-correlated relaxation[3] to obtain information about dihedral angles, distances, and projection angles, respectively. If interconversion of conformers takes place and is fast on the NMR time scale, NMR spectroscopic parameters for flexible parts of the molecule are motionally averaged. This effect is one of the main complications in the structure determination of nonrigid molecules and often prohibits the determination of the relative configuration of organic compounds.

It has been shown that residual dipolar couplings (RDCs) can yield information complementary to that obtained from ^3J coupling constants and NOE parameters also for organic compounds[4–10] and enable the assignment of relative configurations even in the presence of a limited degree of motion.[11–15] The problem of the joint treatment of an unknown configuration and conformational averaging when residual dipolar couplings are used in structure determination has scarcely been tackled. Herein two approaches are discussed, and it is shown that even conformer populations can be obtained from experimentally determined RDCs.

The example chosen for illustration is a five-membered-ring compound investigated recently by some of us. The α-methylene-γ-butyrolactone had been synthesized as single diastereoisomer,[16] the relative configuration of which (*trans*, denoted **1**, or *cis*, denoted **2**) was unknown and could not be determined by using conventional NMR spectroscopic parameters.[11] By using RDCs, however, it was possible to assign the relative configuration as *trans* (Scheme 1).[11]



and enantiomer

1

Scheme 1. Structure of the α-methylene-γ-butyrolactone **1** with atom numbering.

The number of ring conformers of **1** is restricted to two, denoted A and B in the following. These conformers are envelope conformations, one with C2 below (A) and one with C2 above (B) the almost planar arrangement of the remaining ring atoms (see also Figure SI1 in the Supporting Information).[17] In the previous study,[11] we used the transition structure between A and B as a crude approximation of the average ring conformation of **1**. Additionally, we fitted the structures of the two rigid conformers to the RDC data by using one order tensor[18,19] each. By using this method we were able to assign the relative configuration as *trans*.[20] However, we did not attempt to extract information concerning the populations of the two conformers (p_A and p_B), which was therefore one subject of the current investigation.

The direct (residual) dipolar coupling D_{IS} between spins I and S, with magnetogyric ratios γ_I and γ_S, is given by Equation (1):[9,21]

$$D_{IS} = \left\langle b_{IS}\left(\frac{3\cos^2\Theta_{IS} - 1}{2}\right)\right\rangle \tag{1}$$

$b_{IS} = -\frac{\mu_0\gamma_I\gamma_S\hbar}{8\pi^2 r_{IS}^3}$ is the dipole–dipole coupling constant (in Hz), Θ_{IS} is the angle between the interspin vector and the external magnetic field, and r_{IS} is the interspin distance (which corresponds to the bond length for directly bound nuclei). The angular brackets indicate that the RDCs are averaged over both molecular tumbling and internal motion.

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[**] C.M.T. thanks Prof. M. Reggelin for his support, Dr. F. Delaglio for discussions in the initial stages of the project, and the Deutsche Forschungsgemeinschaft (TH1115/2-1 and Emmy Noether Programme, TH1115/3-1) for funding. R.B. acknowledges financial support by the Volkswagen Foundation as well as computer time provided by the Center for Scientific Computing (CSC) Frankfurt. I.L. thanks MEC for a FPU travel grant. This research was supported by grants (to A.M.) from the Swedish Research Council and the Carl Trygger Foundation. C.M.T and A.M. thank Prof. Veracini for his encouragement.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200902398.

For flexible compounds, both r_{IS} and Θ_{IS} become conformation-dependent.[22] It is therefore necessary to examine whether the internal motion can be approximately separated from the reorientation of the molecule and how the separation of these processes can be incorporated into the analysis of RDCs. As discussed by Burnell and de Lange,[23,24] the mode of separation of reorientation and conformational interconversion depends on the timescales of the two processes. If conformational interconversion is fast relative to molecular reorientation, it is sufficient to use one common order tensor for all conformers. If reorientation and conformational interconversion occur on similar time scales, or conformational interconversion is slow relative to reorientation, one order tensor for each conformer is required.

The previously reported[11] 22 experimental RDCs were analyzed in two ways: 1) We extracted the population by superimposing the two conformers (in a physically sound way) and by determining one common order tensor; 2) we determined independent tensors for each conformer and searched for a criterion for the extraction of the conformer population. This second approach is less approximate.

The inherent assumptions are that the conformational equilibrium can be described by an ensemble of rigid conformers; thus, internal motion and reorientation are completely independent and can therefore be separated.[23,25,26] It is also assumed that the conformational equilibrium is not (significantly) shifted by the orienting medium.[27] Our observations, which seem to justify this assumption, are discussed below, first for the common-order-tensor approximation and then for the multiple-tensor approach.

The use of a common order tensor requires the physically meaningful assignment of a molecule-fixed-axis system to the various conformer structures to enable approximate separation of the internal motion and reorientation.[28] One approach is to view the structures of the conformers as vibrationally displaced from one rigid reference arrangement to which the molecular frame is attached in such a way that its origin resides at the center of mass and its axes are oriented in a predefined manner (e.g. coinciding with the principal axes of inertia). Subsequently, the molecule-fixed coordinate system is assigned to the distorted structures so as to diminish the coupling between reorientation (rotation, tumbling) and internal motion (vibration). This task is frequently encountered in theoretical rovibrational spectroscopy of rigid molecules, whereby the coupling between vibrational and rotational motion in the kinetic energy is minimized when the Eckart conditions are satisfied.[29–31] Burnell and de Lange suggested that the Eckart conditions may also be employed for the analysis of (residual) dipolar couplings[23] when conformational equilibria are approximately accounted for. This possibility has, however, apparently not been considered in more recent applications of RDCs of weakly aligned molecules.

We show herein that satisfied Eckart conditions for the superposition of conformers of **1** enable assignment of the relative configuration and even extraction of the conformer population. We used the program hotFCHT[32,33] to perform the Eckart transformation. The resulting Cartesian coordinates for the two conformers of each diastereomer, *trans*-configured **1** and *cis*-configured **2**, in the Eckart system are given in the Supporting Information.

If a common order tensor S for all conformers is assumed, Equation (1) can be expressed as follows [Eq. (2)]:[34]

$$D_{IS} = \sum_{i\in\{\mathrm{A,B}\}} \sum_{\alpha,\beta\in\{x,y,z\}} p_i b_{IS}^{(i)} \cos\theta_\alpha^{(i)} \cos\theta_\beta^{(i)} S_{\alpha\beta} \tag{2}$$

in which $\theta_\alpha^{(i)}$ is the angle between the interspin vector in the ith conformer and the molecule-fixed α axis ($\alpha,\beta\in\{x,y,z\}$), p_i is the population of the ith conformer (with $\sum_i p_i = 1$), and $S_{\alpha\beta}$ are the (in this approach only) five linearly independent order parameters required[35] to describe the partial alignment of the molecular frame. In the study of the present compound, only the two conformers A and B were considered (i.e. $i\in\{\mathrm{A,B}\}$). This approach can, however, be readily extended to more than two conformers.

The strategy used in the analysis was to fit the experimental coupling constants collected in Table SI1 of the Supporting Information to Equation (2)[36] with directional cosines taken from the previously reported energy-minimized structures computed ab initio. The order parameters were fitted, whereas the population p_A was varied systematically in small steps. In this approximation, both the order tensor and the population can be determined, and the order parameters in Equation (2) are different for every value of p_A. Numerical fitting was performed by using a computer code written in-house that minimizes the factor q, which is given by Equation (3):[37]

$$q = \sqrt{\frac{\sum_{I>S}^{N} [(D_{IS}^{\mathrm{calc}} - D_{IS}^{\mathrm{exp}})/\sigma_{IS}^{\mathrm{exp}}]^2}{\sum_{I>S}^{N} [D_{IS}^{\mathrm{exp}}/\sigma_{IS}^{\mathrm{exp}}]^2}} \tag{3}$$

in which D_{IS}^{calc} and D_{IS}^{exp} are the calculated and experimental RDCs, respectively, $\sigma_{IS}^{\mathrm{exp}}$ is the experimental standard deviation, and N is the number of measured RDCs. The sum runs over the various nonidentical combinations of I and S. As can be seen from Equation (3), a small q factor indicates a good numerical fit.

The same population can also be obtained by solving the system of equations with singular value decomposition (SVD),[38] as implemented in a development version[39] of hotFCHT. Nonlinear strategies would be required, however, to fit both $\{S_{\alpha\beta}\}$ and p_i simultaneously.[15]

The population dependence of q is displayed in Figure 1. Clearly, there is no population for *cis*-configured **2** for which q is lower than for **1**. This result implies that the relative configuration can safely be assigned as *trans*. Furthermore, despite the fact that the population dependence of q is rather weak, a shallow minimum is observed for q at $p_\mathrm{A} = 0.6(2)$ for compound **1**, with $q = 0.095$.[40] Thus, an ensemble constructed from conformer A and conformer B in a 60:40 ratio fits the experimental data better than any conformer alone.

Considering the serious approximations introduced when a single order tensor is used for both conformers, it is surprising to find that the population is in excellent agreement

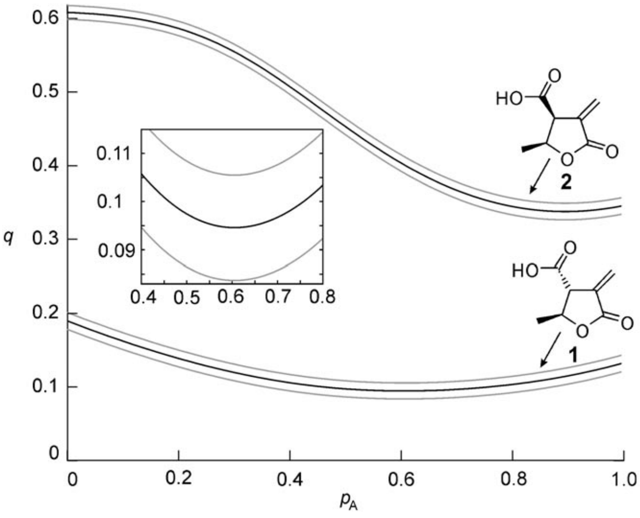

Figure 1. Population dependence of q for **1** (lower, bold line and inset) and **2**, as determined by using one common order tensor and 22 RDCs. The region around the minimum ($p_A = 0.6(2)$ at $q = 0.095$) for **1** is shown in the inset. The value in brackets and the gray lines indicate one standard deviation, as determined from 1000 Monte Carlo steps.[38,52] A cross-validation procedure is shown in Figure SI4 in the Supporting Information.[40]

with the values extracted from J coupling constants in isotropic solution ($p_A = 0.6(2)$; see the Supporting Information) and with the population calculated from the energy difference between the conformers as computed at the MP2 level of theory (p_A ca.0.6).[11]

A more general way to treat conformational equilibria is to use one separate order tensor for each conformer.[11,23,24] In this approach, five (linearly independent) order parameters for each conformer need to be determined[35] by using Equation (4):

$$D_{IS} = \sum_{i\in\{A,B\}} \sum_{\alpha,\beta\in\{x,y,z\}} p_i b_{IS}^{(i)} \cos\theta_{\alpha}^{(i)} \cos\theta_{\beta}^{(i)} S_{\alpha\beta}^{(i)} \qquad (4)$$

in which $S_{\alpha\beta}^{(i)}$ are the order parameters of the ith conformer (A and B in this case).[41]

Again we assumed that a lower q factor indicates a better fit.[42] In this approach, however, the population parameter is not linearly independent of the (10) order parameters. Therefore, the value of q remains constant when the population is scanned, and constraints must be introduced to determine the populations of conformers. The population and the magnitude of the order tensor are not linearly independent fit parameters in this approach. This magnitude is usually defined as the generalized degree of order, ϑ [Eq. (5)]:[19,43,44]

$$\vartheta^{(i)} = \sqrt{\frac{2}{3}\sum_{\alpha,\beta\in\{x,y,z\}} \left(S_{\alpha\beta}^{(i)}\right)^{2}} \qquad (5)$$

in which $i\in\{A,B\}$ enumerates the two conformers. For the following step, we thus introduced the assumption that the orienting media align the two conformers A and B with equal strength. This approach seems to be justified, as the interactions between the solute and the medium used in this study (pentaethylene glycol monodecylether ($C_{12}E_5$)/n-hexanol/D_2O) are weak.

We require that the ϑ value of the two conformers is equal: $\vartheta^A = \vartheta^B$. The population scan of the two generalized degrees of order is shown in Figure 2. The intersection of the curves yields the population of the conformational equilibrium. Since $q_{cis} > q_{trans}$ ($q_{cis} = 0.098(10)$ and $q_{trans} = 0.049(10)$), the relative configuration can safely be assigned as *trans*. Therefore, only the *trans*-configured diastereoisomer **1** was considered.

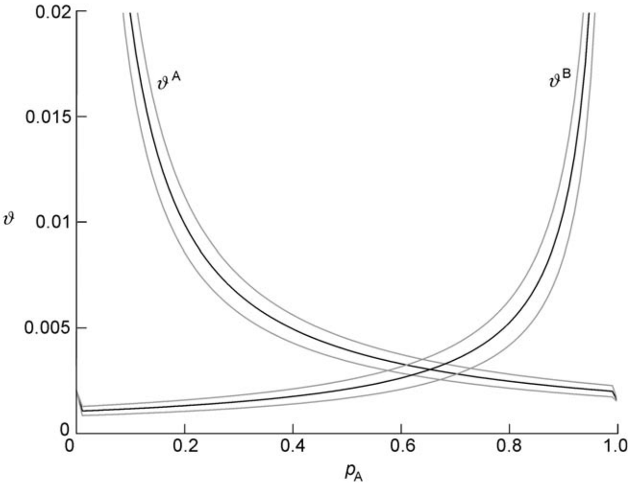

Figure 2. Population dependence of the generalized degrees of order for **1**, as calculated by using 22 RDCs and one order tensor for each conformer, $\vartheta^A$and ϑ^B. The gray lines indicate one standard deviation, as determined from 1000 Monte Carlo steps.[38,52] A cross-validation procedure is shown in Figure SI5 in the Supporting Information.[40]

The curves intersect at $p_A = 0.65(7)$, which indicates that a 65:35 ratio of conformer A to conformer B is in agreement with the RDC data observed.[40] In this approach, the error in the extracted population is much smaller (one standard deviation is 0.07) than that for the single-tensor fit (one standard deviation is 0.20). Again, the value extracted from RDCs by using the multiple-conformer multiple-tensor fit is well within experimental estimates of the population determined from calculated J coupling constants, from quantum-chemical calculations, and by the multiple-conformer single-tensor fit described above. The orientations of the tensors obtained from the multitensor fit, however, do not coincide.

The drawback of the multiple-conformer multiple-tensor approach is that five order parameters[35] need to be determined for each conformer. Increased molecular flexibility (an increased number of conformers) leads to a situation in which the number of parameters that must be extracted from the analysis can clearly exceed the experimental data set, so that systems become undetermined. This factor restricts the use of this approach to moderately flexible molecules. When molecules with increased flexibility are investigated, analytical models that allow for continuous bond rotation might be required. Three approaches that have frequently been used for the interpretation of (R)DCs are the additive potential (AP) model,[45] the maximum entropy (ME) approach,[46] and a combination of these two strategies. The last method was developed for low-order liquid crystals and is denoted APME.[47–51]

In summary, we have presented two approaches, namely, I) the use of a single effective order tensor and II) a less approximate approach in which each conformer is accounted for by an individual order tensor, for the analysis of the relative configuration and conformational equilibrium of a moderately flexible molecule, the α-methylene-γ-butyrolactone **1.** This compound exhibits two major ring conformations, and the RDCs (and other NMR spectroscopic parameters) are averaged by motion that is fast on the NMR time scale.

Both approaches enabled the simultaneous determination of configuration (*trans* in this case) and conformer population. The populations extracted for the α-methylene-γ-butyrolactone investigated in this study by both methods fit the populations obtained from (calculated) J coupling constants and quantum-chemical calculations very well. This good agreement also indicates that the conformational equilibrium is not shifted (at least not significantly) by the orienting medium. Another way to investigate whether or not the orienting medium influences the conformational equilibrium would be to compare data extracted from different orienting media.[26] A study of this type is currently in progress.

When the analysis is performed with a single order tensor, one has to bear in mind that the approximation made, namely, that all conformers can be described by one common order tensor, is relatively crude. We employed the Eckart conditions[23,29,31] to superimpose the individual conformers. For the molecule in this study, it seems to be reasonable to make this approximation of one common order tensor, as both the relative configuration and the conformer population could be extracted, and qualitatively the same information was obtained as in the individual-order-tensor fit.

As to the general applicability of the approaches discussed herein, the simultaneous assignment of relative configuration and conformer population is not guaranteed to be possible for other compounds, and even wrong assignments are not a priori ruled out. Methods such as sensitivity analysis or additional physically justified constraints in the multitensor approach may provide useful information and are thus under investigation.[39]

Received: May 5, 2009

Keywords: Eckart conditions · motional averaging · NMR spectroscopy · residual dipolar coupling · structure elucidation

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[17] This nomenclature of course depends on the absolute configuration. Herein we refer to the structures as depicted in Scheme 1.

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[21] Equation (1) has been modified from that given in: M. H. Levitt, *Spin Dynamics: Basics of Nuclear Magnetic Resonance*, Wiley, New York, **2001**, pp. 425 – 431. The distance r_{IS} was moved into the "averaging bracket" so that it was also possible to account for intramolecular flexibility.

[22] In principle, r_{IS} is also averaged by small-amplitude vibrational motion, which would require additional vibrational corrections. This motion is usually neglected when dealing with RDCs; see, however, Refs. [23,24] and references therein.

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[34] Equation (2) has the same meaning as that presented in Ref. [12]. The population used in that study, however, was extracted from J coupling constants and was not varied.

[35] Owing to symmetry, the number of independent order parameters can also be smaller than five.

[36] To ensure that we had well-defined order parameters, we checked that the rank of each directional cosine matrix was five (number of order parameters) and that its condition number was sufficiently small. These criteria were fulfilled for both diastereoisomers (**1** and **2**).

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[40] A cross-validation procedure was performed by removing in turn each of the 22 RDCs and performing the analysis with the remaining 21 RDCs. The results are shown in Figures SI4 (common-order-tensor approximation) and SI5 (multitensor approximation) in the Supporting Information.

[41] In practice, the parameters are determined by constructing one large S vector containing all linearly independent order parameters in one column.

[42] We again verified that enough independent RDCs were available by checking that the rank of the (combined) directional cosine matrix was five times the number of conformers (ten for the two conformers used in this study) and that its condition number was sufficiently small. These criteria were fulfilled for both diastereoisomers (**1** and **2**).

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